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(54) Title: ORGAN TRANSPLANT SOLUTIONS AND METHOD FOR TRANSPLANTING AN ORGAN

(57) Abstract

There is provided cardioplegic solutions for arresting an organ intended for transplantation and preservation solutions for perfusing and storing an organ while awaiting transplantation. The cardioplegic solutions include, per liter of solution, a balanced isotonic solution of sodium, potassium, calcium, and magnesium ions and bicarbonate in a physiologically acceptable amount, at least 0.5 μM of an amiloride-containing compound; and water sufficient to make a liter of solution. The preserving solutions include per liter of solution, a balanced isotonic solution of sodium, potassium, calcium, and magnesium ions and bicarbonate in physiologically acceptable amount, from about 1.0 μM to about 5.0 μM of an amiloride-containing compound; and water to make a liter of solution. In addition the preservation solution may contain other components such as EDTA, a small amount of adenosine, and at least one antioxidant. The amiloride-containing compound may be amiloride, hexamethylene amiloride, dimethyl amiloride, ethyl isopropyl amiloride or methyl isobutyl amiloride. There is also provided a method for arresting an organ, storing an organ and transplanting an organ all at room temperature for up to at least 24 hours.

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ORGAN TRANSPLANT SOLUTIONS AND METHOD FOR TRANSPLANTING AN ORGAN

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to cardioplegic solutions, to organ preservation solutions, and to methods for transplanting organs. More particularly, this invention relates to cardioplegic solutions for arresting an organ for transplantation, to preservation solutions for perfusing and storing an organ while awaiting implantation, and to methods for using the cardioplegic and preserving solutions during transplantation of an organ.

The Prior Art

A great deal of research progress has been made over the years in understanding cellular mechanisms, as well as developing new transplantation techniques for keeping organs viable not only during storage but also after reperfusion of these organs. As a result, organ transplantation, including heart transplantation, is an established elective operation. A significant factor limiting the clinical application of organ transplantation is the deviation of viability of the organ after removal from the donor.

At present, the two most frequently used methods for heart transplantation are simple hypothermic storage and continuous pulsatile perfusion. With simple hypothermic storage, the heart is arrested with a cardioplegic solution, then removed from the donor and cooled rapidly. This is usually achieved by a combination of cooling and a short period of perfusion to drop the heart temperature as quickly as possible to a temperature between 0°C. and 4°C. where

it may be held up to about 6 hours. While cold storage enables organs to be transplanted, the time during which the organ is viable is short. Cold storage decreases the rate at which intracellular enzymes, essential cellular components necessary for organ viability, degrade but does not stop metabolism.

The second method of organ preservation which has undergone extensive investigation, continuous pulsatile perfusion, includes the following steps: (1) pulsatile flow, (2) hypothermia, (3) membrane oxygenation, and (4) a perfusate containing both albumin and lipids. Although being more technically complex and costly, the advantages to using continuous pulsatile perfusion over simple hypothermia include longer viability of the organ and viability testing prior to implantation.

The compositions of numerous cardioplegic and preservation solutions have been extensively studied. For example, the protective properties of three cardioplegic solutions were compared by Galiñanes et al. (M. Galiñanes, T. Murashita and D.J. Hearse (1991) *The Journal of Heart and Lung Transplantation* (11) 624-635, at low temperatures and short time periods. A comparison of cold preservation solutions was set forth in G. Tian et al. (1991) *The Journal of Heart and Lung Transplantation* (10) 975-985, where the cold preservation solutions limited the storage time of the organs.

A storage solution for preserving organs which can be used at temperatures from 0°C. to 37°C. but was limited in storage time was disclosed in U.S. Patent No. 5,145,771 to Lemasters et al. The solution requires the use of the colloid, hydroxyethyl starch, for oncotic support against interstitial edema. In the present invention edema is not a problem because no oxygen-derived free radicals are available to injure the organ.

Preserving organs at between 0°C. and 4°C. results in damage to the organ during storage and upon reperfusion with a warm reperfusion solution. Injury to the organ occurs through the loss of endothelial cells due to dissolved oxygen in the reperfusion solution. Although some of the solutions of the prior art have been useful to extend the storage time of donor organs and lessen injury to the organ upon reperfusion, cell injury still occurs. Therefore, it is desirable to extend the viable organ life and improve the quality of the transplanted organ. Extending the organ viability allows sufficient time for compatibility testing of the donor and recipient and increased organ availability.

The recovery of cardiac function is also greatly influenced by the time lapse between removal from the donor and reperfusion as well as the efficacy of protective interventions used during that period. To overcome the deleterious effects of ischemia, techniques such as intermittent or continuous perfusion have been used. Finally, reperfusion itself, although necessary for the survival of the tissue, may initiate a series of events known as reperfusion induced injury, which, if occurring, limit the extent or rate of recovery. Thus, modification of the nature of reperfusion is desirable to improve the recovery of the ischemic/reperfused myocardium.

More particularly, as a result of the deprivation of circulation, and thus oxygen (i.e., ischemia) during transplantation, the sodium pump, which normally maintains the intracellular composition high in potassium, magnesium, and phosphate and low in sodium and chloride, ceases to function due to the lack of energy, resulting in an inflow of sodium and chloride into the cells, and an outflow of potassium and to a lesser extent magnesium from the cells. The result of these rapid changes in Na^+ - H^+ distribution

in the cell is a net gain, not merely an exchange, of intracellular ions followed by water and a profound loss of potassium and magnesium resulting in damage to the organ. The protective effects of Na/H exchange inhibitors, including amiloride and its analogs in the reperfused myocardium has been studied by Moffat, et al. (M. P. Moffat and M. Karmazyn, (1993), *J. Molec. Cell Cardiol* (25), 959-971).

It is therefore the general object of the present invention to provide preserving solutions for pulsating and storing organs while awaiting implantation which inhibits ion exchange, extends the vitality of the organ, and reduces damage to the cells.

Another object of the present invention is to provide a method for arresting and preserving organs which extend the maximum life of the organ during transplantation.

Yet another object of the present invention is to provide a method of transplanting organs in which storage of the organ may be carried out at room temperature for up to at least 24 hours without significant damage to the organ.

Other objects, features, and advantages of the invention will be apparent from the following details of the invention as more fully described.

SUMMARY OF THE INVENTION

In accordance with these objects and the principles of this invention, there are disclosed cardioplegic solutions and preserving solutions for use in the transplantation of organs, and to methods for transplanting organs using the solution in combination with cardioplegic solutions, which methods increase storage times and lessen injury to the organ.

It has been found that a cardioplegic solution containing an amiloride-containing compound is effective in achieving the objectives of this

invention. In one aspect of this invention, the cardioplegic solution includes a balanced isotonic solution including sodium, potassium, calcium and magnesium ions and bicarbonate in a physiologically acceptable amount, at least 0.5 μM, preferably from about 1.0 μM to about 5.0 μM, and most prefereably 1.0 μM to 3.0 μM of an amiloride-containing compound, and water sufficient to make a liter of solution.

The cardioplegic solution also preferably contains glucose to enhance organ preservation, adenosine to prevent fibrillation of the organ prior to removal from the donor, and EDTA as a chelating agent. Optionally, the cardioplegic solution contains heparin and at least one antioxidant.

The preservation solution, while similar to the cardioplegic solution in starting composition in that it is based on a balanced isotonic solution including sodium, potassium, calcium, magnesium ions and bicarbonate in a physiologically acceptable amount also includes from 1.0 μM to about 5.0 μM of an amiloride-containing compound.

The preservation solution preferably includes at least one antioxidant, such as, dimethyl thiourea (DMTU), catalase as a hydrogen peroxide scavenger and apoferritin to decrease iron content within the preservation solution. Since the organ has been arrested by the cardioplegic solution, the preservation solution includes less adenosine and heparin is not normally needed. In addition, the preservation solutions optionally may include hormones, such as insulin and prostaglandin and antibiotics.

The inclusion in the isotonic solution of both the cardioplegic solution and the preservation solution of an amiloride-containing compound may be amiloride itself, or amiloride analogs, such as hexamethylene amiloride (HMA), dimethyl amiloride (DMA), ethyl isopropyl amiloride (EIPA), or methyl

isobutyl amiloride (MIA), all of which inhibit the NA^+ - H^+ exchange in the organ cells. Dimethyl amiloride is particularly preferred.

The invention also provides a method for transplanting an organ which includes steps for arresting and removing the organ from the donor, and for preserving and storing the organ intended for implantation. The method of the invention includes arresting the organ to be donated with a cardioplegic solution. The organ is removed and connected to a perfusion apparatus where it is maintained at a temperature between about 0°C. to about 37°C., preferably from about 15°C. to about 25°C. while perfusing with the preservation solution. Thus, the novel features of the present invention include storing the organ at warm temperatures, i.e., up to about room temperature, while perfusing the organ. It is believed that the ability to store the organ at or near room temperature prevents mechanical damage that can result from cold storage, and that continuous perfusion with the preservation solution maintains the organ's metabolic requirements and avoids potential metabolic blocks. As a result, storage times for the organ can be increased up to at least 24 hours.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to new cardioplegic solutions for arresting an organ intended for transplantation and to new preservation solutions for storing and perfusing organs intended for implantation in a patient requiring such implant. Suitable organs on which the solutions of this invention may be used include, for example, heart, liver, kidney and pancreas.

The individual components of the present invention are all nontoxic and have been found to be stable during storage. While some of the components of

the present invention are similar to those of other known cardioplegic and preservation solutions, it has surprisingly been found that the addition of amiloride or an amiloride-containing compound to a balanced isotonic solution including sodium, potassium, calcium, magnesium, and bicarbonate ions in a physiologically acceptable amount to form a cardioplegic solution and its use with the preservation solution of the present invention allows organs to be preserved at room temperature for at least 24 hours without significant damage to the organ.

Both the cardioplegic solutions and the preservation solutions of the present invention are based on a balanced isotonic solution including sodium, potassium, calcium and magnesium ions as well as glucose and sodium bicarbonate in a physiologically acceptable amount. Certain of these types of solutions are well known, such as the one described below, known as Krebs-Henseleit-bicarbonate solution, which has the following composition:

TABLE 1

	Concentration Ranges in 1 Liter		
NaCl	85.0 mM	to	145 mM
KCl	3.0 mM	to	30 mM
$CaCl_2$	0.5 mM	to	2.5 mM
KH_2PO_4	0.7 mM	to	1.3 mM
$MgSO_4$	0.9 mM	to	4.8 mM
$NaHCO_3$	15.0 mM	to	35 mM
Glucose	1.0 mM	to	50 mM

The Cardioplegic Solution

The cardioplegic solution is made by starting with the balanced isotonic solution described above. The amount of potassium chloride in the cardioplegic solution is preferably from about 20 mM to about 30 mM.

To the balanced isotonic solution is added an amiloride-containing compound in an amount of at least 0.5 μM, preferably from about 1.0 μM to about 5.0 μM, and most preferably from 1.0 μM to 3.0 μM. By amiloride-containing compound, it is meant to include amiloride and amiloride analogs. The addition of amiloride, designated chemically as 3,5-diamino-6-chlor-*N*-(diaminomethylene) pyrazinecarboxamide monohydrochloride has been found to inhibit the $Na^+ - H^+$ exchange. Other amiloride-containing compounds or analogues which may be used in the cardioplegic solution include, for example, hexamethylene amiloride (HMA) designated chemically as 5-(*N*,*N*-hexamethylene)-amiloride, dimethyl amiloride (DMA) designated 5-(*N*,*N*-dimethyl)-amiloride, ethyl isopropyl amiloride (EIPA), designated 5-(*N*-ethyl-*N*-isopropyl)-amiloride, and methyl isobutyl amiloride (MIA), designated 5-(*N*-methyl-*N*-isobutyl)-amiloride. (Merck Sharpe & Dohme, West Point, Pa).

To be effective, the cardioplegic solution needs to prevent fibrillation of the organ in a relatively short period of time, e.g., 2 minutes to 5 minutes or less. For that purpose, adenosine is added to the solution in an amount from about 5 μM to about 15 μM per liter, preferably about 10 μM per liter. Adenosine rapidly arrests the heart (within seconds), improves the preservation properties and increases the glucose uptake.

The cardioplegic solution also preferably includes ethylene diaminetetraacetic acid (EDTA) in an amount from 0.5 mM to about 1.5 mM as a chelating agent. The solution also may optionally contain other ingredients, such as at least one antioxidant, for example, catalase, in an amount effective to inhibit the generation of oxygen-derived free radicals via hydrogen peroxide.

Heparin is used in carrying out the method of this invention, and may be included either directly in the cardioplegic solution or it may be administered to the donor organ separately. The addition of heparin in an amount of from about 500 units to about 1500 units, preferably 1000 units is required to prevent blood clots from forming within the coronary arteries during cardioplegic arrest and excision prior to organ storage and implantation.

The Preservation Solution

The preservation solution is designed to prevent various mechanisms which cause injury to the organ and thus must be a composition that (1) prevents or restricts intracellular acidosis, (2) prevents the expansion of intracellular space, (3) prevents injury from oxygen-derived free radicals, especially during reperfusion, (4) enables the regeneration of high-energy phosphate compounds during reperfusion, (5) sustains appropriate metabolic requirement, and (6) prevents the rapid changes in intracellular Na^+-H^+-Ca^{++} following reperfusion.

While the cardioplegic solution and the preservation solution begin with the balanced isotonic solution described above there are significant differences in the final compositions. For example, the preservation solution begins with the isotonic solution, wherein the potassium concentration is maintained at preferably from 3.0 mM to about 8.0 mM. Magnesium chloride may be used in place of potassium chloride.

To the balanced isotonic solution is added an amiloride-containing compound in an amount from about 1.0 μM to about 5.0 μM, preferably 1.5-3.0 μM. By amiloride-containing compound, it is meant to include amiloride and amiloride analogs. The addition of amiloride, designated chemically as 3,5-diamino-6-

chlor-*N*-(diaminomethylene) pyrazinecarboxamide monohydrochloride has been found to inhibit the Na^+ - H^+ exchange. Other amiloride-containing compounds or analogues which may be used in the cardioplegic solution include, for example, hexamethylene amiloride (HMA) designated chemically as 5-(*N*,*N*-hexamethylene)-amiloride, dimethyl amiloride (DMA) designated 5-(*N*,*N*-dimethyl)-amiloride, ethyl isopropyl amiloride (EIPA), designated 5-(*N*-ethyl-*N*-isopropyl)-amiloride, and methyl isobutyl amiloride (MIA), designated 5-(*N*-methyl-*N*-isobutyl)-amiloride. (Merck Sharpe & Dohme, West Point, Pa).

While adenosine is included in the preservation solution, the amount of adenosine is considerably less than the amount of adenosine present in the cardioplegic solution because the organ has been previously arrested. The amount of adenosine in the preservation solution is normally from 0.7 μM to about 2.0 μM, preferably about 1.0 μM.

The preservation solution also preferably includes ethylene diaminetetraacetic acid (EDTA) in an amount from 0.5 μM to about 1.5 μM as a chelating agent. It has also been found desirable to add from 10 ηM to about 100 ηM of caprylic acid which helps the solution to bypass blocked fatty acid utilization and from 10 μg/L to 100 μg/L of apoferritin which serves to eliminate iron (Fe^{++}) which causes breakdown of the cells. Desferrioxamine may also be used to chelate the iron. Dichloroacetic acid may be employed to reduce lactate.

Suitable antioxidants include, but are not limited to, allopurinol, glutathione, beta-carotene, catalase, superoxide dismutase, dimethyl thiourea (DMTU), diphenyl phenylene diamine (DPPD), mannitol or cyanidanol in an amount effective to inhibit the generation of oxygen-derived free radicals. The antioxidants are present in an amount from 1 ηM to 10

ηM. Antibiotics may be added for transplantable organs, but is not generally added during acute studies.

Method of Use

The transplantation method of the present invention is to arrest the organ using the cardioplegic solution, preserve and store the organ with the preservation solution and reperfuse with the preservation solution.

In a preferred method, sufficient cardioplegic solution is injected to arrest, for example the heart and prevent fibrillation. The surgeon then removes the organ and connects the heart to a perfusion apparatus comprising tubing and pumps. The preservation solution is then perfused through the heart while gassed with oxygen and carbon dioxide while it is awaiting implantation into a patient. A perfusion rate of 50 mL/hour at 1.0°C. has been found to be effective. The method of perfusing the heart can be at either a constant flow or pressure.

The solution can be used at all temperatures ranging from 0°C. to normal body temperature, 37°C. At temperatures of from about 12°C. to about 37°C., the solution is more protective than other known preservation solutions. Unlike other storage solutions, it continues to be protective above 10°C. for at least 24 hours.

The following examples are provided to further illustrate the present invention and are not to be construed as limiting the invention in any manner.

Example 1

A liter of cardioplegic solution having the following composition was prepared.

NaCl	118	mM
KCl	30	mM
$CaCl_2$	1.75	mM
KH_2PO_4	1.2	mM
$MgSO_4$	1.2	mM
$NaHCO_3$	25	mM
Glucose	11	mM
Adenosine	10	μM
EDTA	1.0	mM
DMA	1.0	μM
Heparin	1000	units
Distilled, deionized water	q.s.	

Into a 1000 mL volumetric flask the Krebs-Henseleit solution was added and double distilled water was added to make one liter while stirring. The rest of the components were added one at a time. After all the components were added the pH was adjusted to 7.3 with NaOH and the flask was gassed with O_2/CO_2:95/5%. The solution was stirred for about thirty minutes and filtered to remove any undissolved particles (5.0 μ porosity filter). After sterile filtration the solution was ready to use.

Example 2

A liter of preservation solution having the following composition was prepared.

NaCl	118	mM
KCl	4.7	mM
$CaCl_2$	1.75	mM
KH_2PO_4	1.2	mM
$MgSO_4$	1.2	mM
$NaHCO_3$	25	mM
Glucose	11	mM
Adenosine	1.0	μM
EDTA	1.0	mM
Apoferritin	100	μg/L
Catalase	10	μg/L
DMTU	10	ηM
DPPD	10	ηM
Caprylic Acid	50	ηM
DMA	3.0	μM
Insulin	200.0	μ Units/mL
Distilled, deionized water	q.s.	

The preservation solution was prepared in much the same manner as the cardioplegic solution of Example I, that is, by adding a Krebs-Heneseleit solution to a 1000 mL volumetric flask with double distilled water to make one liter while stirring. The rest of the components were added one at a time and the pH of the solution was adjusted to about 7.3 with NaOH and gassed with 95% oxygen plus 5% carbon dioxide. The solution was stirred for about thirty minutes and filtered to remove any undissolved particles (5.0 μ porosity filter). After sterile filtration the solution was ready to use.

Example 3

A female mongrel dog weighing about 20 kg. was anesthetized. An IV hydrating solution of 5.0% dextrose in 0.45% saline at 75 cc/hr was given throughout the procedure. The heart was exposed by a sternotomy. The cardioplegic solution of Example 1 was administered to arrest the heart, the heart was then excised. The heart was placed in ice and promptly transferred to the laboratory and placed in a perfusion apparatus at room temperature where the aorta was attached to a tube for continuous perfusion with the preserving solution of Example 2. After 2-3 minutes the heart started beating at a pulse rate of 50 beats per minute. Excess preserving solution was allowed to fill the container until the heart was covered to provide buoyancy for the heart so as not to injure the aorta. Perfusion with the solution from Example 2 continued throughout the storage time. The heart continued to beat for over 24 hours.

This experiment demonstrates that the cardioplegic and preservation solutions and methods of the present invention increase the preservation time between harvesting an organ and transplantation and allows the organ to remain at room temperature without serious degradation of the organ cells.

The present invention has been described in detail and with particular reference to the preferred embodiments. Those skilled in the art will appreciate that changes can be made without departing from the spirit and scope thereof. Accordingly, the present invention is to be defined by the following claims, with equivalents of the claims to be included therein.

WHAT IS CLAIMED IS:

1. A cardioplegic solution for arresting an organ selected from the group consisting of the heart, liver, kidney and pancreas intended for transplantation comprising, per liter of solution:

(a) a balanced isotonic solution comprising sodium, potassium, calcium, magnesium and bicarbonate ions in a physiologically acceptable amount;

(b) at least 0.5 μM of an amiloride-containing compound; and

(c) water.

2. The carioplegic solution according to Claim 1, wherein said amiloride-containing compound is a compound selected from the group consisting of amiloride, hexamethylene amiloride, dimethyl amiloride, ethyl isopropyl amiloride and methyl isobutyl amiloride.

3. The carioplegic solution according to Claim 1, wherein and amiloride-containing compound is dimethyl amiloride.

4. The cardioplegic solution according to Claim 1, further comprising from about 0.5 mM to about 1.5 mM of EDTA.

5. The carioplegic solution according to Claim 1, further comprising from about 5.0 μM to about 15 μM of adenosine.

6. The cardioplegic solution according to Claim 1, further comprising from about 500 units to about 1500 units of heparin.

7. The cardioplegic solution according to Claim 1, further comprising an antioxidant in an amount effective to inhibit the generation of oxygen-derived free radicals.

8. A cardioplegic solution for arresting an organ selected from the group consisting of the heart, liver, kidney and pancreas intended for transplantation comprising, per liter of solution:

NaCl	85 mM to 145 mM
KCl	20 mM to 30 mM
$CaCl_2$	0.5 mM to 2.5 mM
KH_2PO_4	0.7 mM to 1.3 mM
$MgSO_4$	0.9 mM to 4.8 mM
$NaHCO_3$	15 mM to 35 mM
Glucose	1.0 mM to 50 mM
EDTA	0.5 mM to 1.5 mM
Distilled, deionized water	q.s.

and from about 0.5 μM to about 5.0 μM of an amiloride-containing compound.

9. The cardioplegic solution according to Claim 8, wherein said amiloride-containing compound is a compound selected from the group consisting of amiloride, hexamethylene amiloride, dimethyl amiloride, ethyl isopropyl amiloride and methyl isobutyl amiloride.

10. The cardioplegic solution according to Claim 8, wherein said compound is dimethyl amiloride.

11. The cardioplegic solution according to Claim 8, further comprising from about 5.0 μM to about 15 μM of adenosine.

12. The cardioplegic solution according to Claim 8, further comprising from about 500 units to about 1500 units of heparin.

13. The cardioplegic solution according to Claim 8, further comprising an antioxidant in an amount effective to inhibit the generation of oxygen-derived free radicals.

14. A cardioplegic solution for arresting an organ selected from the group consisting of the heart, liver, kidney and pancreas intended for transplantation comprising, per liter of solution:

NaCl	118 mM
KCl	30 mM
$CaCl_2$	1.75 mM
KH_2PO_4	1.2 mM
$MgSO_4$	1.2 mM
$NaHCO_3$	25 mM
Glucose	11 mM
Adenosine	10 μM
EDTA	1.0 mM
Dimethyl amiloride	1.0 μM
Heparin	1000 units
Distilled, deionized water	q.s.

15. A preservation solution for storage and reperfusion of organs intended for implantation comprising, per liter of solution:

(a) a balanced isotonic solution comprising sodium, potassium, calcium, magnesium, bicarbonate ions in a physiologically acceptable amount;

(b) from about 0.7 μM to about 2.0 μM of adenosine;

(c) from about 1.0 μM to about 5.0 μM of an amiloride-containing compound; and

(d) water.

16. The preservation solution according to Claim 15, wherein said amiloride-containing compound is a compound selected from the group consisting of amiloride, hexamethylene amiloride, dimethyl amiloride, ethyl isopropyl amiloride and methyl isobutyl amiloride.

17. The preservation solution according to Claim 15, wherein said amiloride-containing compound is dimethyl amiloride.

18. The preservation solution according to Claim 15, further comprising at least one antioxidant in an amount effective to inhibit the generation of oxygen-derived free radicals.

19. The preservation solution according to Claim 15, further comprising from about 10 ηM to about 100 ηM of caprylic acid.

20. The preservation solution according to Claim 15, further comprising from about 100 μ units/mL to about 500 μ units/mL of insulin.

21. A preservation solution useful for preserving organs for storage and reperfusion of organs intended for implantation comprising, per liter of solution:

NaCl	85 mM to 145 mM
KCl	3.0 mM to 8.0 mM
$CaCl_2$	1.0 mM to 2.0 mM
KH_2PO_4	0.7 mM to 1.3 mM
$MgSO_4$	0.9 mM to 4.8 mM
$NaHCO_3$	15 mM to 35 mM
Glucose	1.0 mM to 50 mM
EDTA	0.5 mM to 1.5 mM
Adenosine	0.7 μM to 1.5 μM

Apoferritin	1 μg/L	to 100 μg/L
Catalase	1 μg/L	to 10 μg/L
Caprylic acid	10 ηM	to 100 ηM
DMTU	1 ηM	to 10 ηM
DPPD	1 ηM	to 10 ηM
Dimethyl amiloride	1 μM	to 5 μM
Distilled, deionized water	q.s.	

22. A preservation solution useful for preserving organs for storage and reperfusion of organs intended for implantation comprising, per liter of solution:

NaCl	118 mM
KCl	4.7 mM
$CaCl_2$	1.75 mM
KH_2PO_4	1.2 mM
$MgSO_4$	1.2 mM
$NaHCO_3$	25 mM
Glucose	11 mM
Adenosine	1.0 μM
EDTA	1.0 mM
Apoferritin	100 μg/L
Catalase	10 μg/L
DMTU	10 ηM
DPPD	10 ηM
Dimethyl amiloride	3.0 μM
Insulin	200.0 μ Units/mL
Caprylic Acid	50 ηM
Distilled, deionized water	q.s.

23. A method for removing an organ selected from the group consisting of the heart, liver, kidney and pancreas from a mammal intended for implantation in a mammal requiring such implantation, said method comprising:

arresting said organ prior to removal from a donor with a solution comprising, per liter of solution:

(a) a balanced isotonic solution comprising sodium, potassium, calcium, magnesium, and bicarbonate ions in a physiologically acceptable amount;

(b) at least 0.5 μM of an amiloride-containing compound; and

(c) water.

24. The method according to Claim 23, in which said amiloride-containing compound is a compound selected from the group consisting of amiloride, hexamethylene amiloride, dimethyl amiloride, ethyl isopropyl amiloride and methyl isobutyl amiloride.

25. The method according to Claim 23, in which said amiloride-containing compound is dimethyl amiloride.

26. The method according to Claim 23, in which said solution further comprises from about 0.5 mM to about 1.5 mM EDTA.

27. The method according to Claim 23, in which sid solution further comprising from about 5.0 μM to about 15 μM of adenosine.

28. The method according to Claim 23, in which said solution further comprising from about 500 units to about 1500 units of heparin.

29. The method according to Claim 23, in which said solution further comprising at least one antioxidant in an amount effective to inhibit the generation of oxygen-derived free radicals.

30. The method according to Claim 23, wherein said organ is a heart.

31. A method for removing the heart from a mammal intended for transportation, said method comprising:

arresting said heart prior to removal from a donor with a solution comprising, per liter of solution:

NaCl	85 mM	to	145 mM
KCl	3 mM	to	30 mM
$CaCl_2$	0.5 mM	to	2.5 mM
KH_2PO_4	0.7 mM	to	1.3 mM
$MgSO_4$	0.9 mM	to	4.8 mM
$NaHCO_3$	15 mM	to	35 mM
Glucose	1.0 mM	to	50 mM
EDTA	0.5 mM	to	1.5 mM
Distilled, deionized water	q.s.		

and at least 0.5 μM of an amiloride-containing compound.

32. The method according to Claim 31, wherein said amiloride-containing compound is a compound selected from the group consisting of amiloride, hexamethylene amiloride, dimethyl amiloride, ethyl isopropyl amiloride and methyl isobutyl amiloride.

33. The method according to Claim 31, wherein said amiloride-containing compound is dimethyl amiloride.

34. The method according to Claim 31, in which said solution further comprising from about 5 μM to about 15 μM of adenosine.

35. The method according to Claim 30, in which said solution further comprising from about 500 units to about 1500 units of heparin.

36. The method according to Claim 30, in which said solution further comprising at least one antioxidant in an amount effective to inhibit the generation of oxygen-derived free radicals.

37. The method according to Claim 30, wherein said organ is a heart.

38. A method for removing an organ from a mammal intended for implantation, said method comprising:

arresting the heart prior to removal from a donor with a solution comprising, per liter of solution:

NaCl	118 mM
KCl	30 mM
$CaCl_2$	1.75 mM
KH_2PO_4	1.2 mM
$MgSO_4$	1.2 mM
$NaHCO_3$	25 mM
Glucose	11 mM
Adenosine	10 μM
EDTA	1.0 mM
Dimethyl amiloride	1.0 μM
Heparin	1000 units
Distilled, deionized water	q.s.

39. A method for the preservation, storage and reperfusion of organs intended for implantation, said method comprising:

perfusing said organ with a solution comprising, per liter of solution:

(a) a balanced isotonic solution comprising sodium, potassium, calcium, magnesium ions and bicarbonate in a physiologically acceptable amount;

(b) from about 0.7 μM to about 2.0 μM of adenosine;

(c) from about 1.0 μM to about 5.0 μM of an amiloride-containing compound; and

(d) water.

40. The method according to Claim 39, wherein said amiloride-containing compound is a compound selected from the group consisting of amiloride, hexamethylene amiloride, dimethyl amiloride, ethyl isopropyl amiloride and methyl isobutyl amiloride.

41. The method according to Claim 39, wherein said amiloride-containing compound is dimethyl amiloride.

42. The method according to Claim 39, in which said solution further comprising at least one antioxidant in an amount effective to inhibit the generation of oxygen-derived free radicals.

43. The method according to Claim 39, in which said solution further comprising from about 100 μ units/mL to about 500 μ units/mL of insulin.

44. The method according to Claim 39, wherein said organ is a heart.

45. The method according to Claim 39, further comprising preserving and storing said organ in said solution at from about 0°C. to about 37°C.

46. The method according to Claim 39, wherein said temperature is from 15°C. to 37°C.

47. A method for the preservation, storage and perfusion of an organ intended for implantation, said method comprising:

perfusing said organ with a solution comprising, per liter of solution:

NaCl	85 mM to 145 mM
KCl	3.0 mM to 8.0 mM
$CaCl_2$	1.0 mM to 2.0 mM
KH_2PO_4	0.7 mM to 1.3 mM
$MgSO_4$	0.9 mM to 4.8 mM
$NaHCO_3$	15 mM to 35 mM
Glucose	1.0 mM to 50 mM
EDTA	0.5 mM to 1.5 mM
Apoferritin	1 μg/L to 100 μg/L
Catalase	1 mg/L to 10 mg/L
Caprylic acid	10 ηM to 100 ηM
DMTU	1 ηM to 10 ηM
DPPD	1 ηM to 10 ηM
Dimethyl amiloride	1 μM to 5 μM
Distilled, deionized water	q.s.

48. The method according to Claim 47, in which said solution further comprising from about 0.7 μM to about 2.0 μM of adenosine.

49. The method according to Claim 47, in which the solution further comprising at least one antioxidant in an amount effective to inhibit the generation of oxygen-derived free radicals.

50. The method according to Claim 47, wherein said organ is a heart.

51. The method according to Claim 47, further comprising perfusing, preserving and storing said organ in said solution at from about 0°C. to about 37°C.

52. The method according to Claim 47, wherein said temperature is from 15°C. to 37°C.

53. A method for the preservation, storage and perfusion of an organ intended for implantation, said method comprising:

perfusing said organ with a solution comprising, per liter of solution:

NaCl	118	mM
KCl	4.7	mM
$CaCl_2$	1.75	mM
KH_2PO_4	1.2	mM
$MgSO_4$	1.2	mM
$NaHCO_3$	25	mM
Glucose	11	mM
Adenosine	1.0	μM
EDTA	1.0	mM
Apoferritin	100	μg/L
Catalase	10	μg/L
DMTU	10	ηM
DPPD	10	ηM
Caprylic Acid	50	ηM
Dimethyl amiloride	3.0	μM
Insulin	200.0	μ Units/mL
Distilled, deionized water	q.s.	

54. A method of transplantation of a mammalian heart, said method comprising:

arresting the heart with a cardioplegic solution prior to removal from a donor;

removing said heart from said donor; and

perfusing said heart while storing at a temperature between 0°C. and 37°C. with a preservation solution comprising, per liter of solution, a balanced isotonic solution comprising sodium, potassium, calcium, and magnesium ions and bicarbonate in a physiologically acceptable amount, from about 1.0 μM to about 5.0 μM of an amiloride-containing compound.

55. The preservation solution according to Claim 54, wherein said amiloride-containing compound is a compound selected from the group consisting of amiloride, hexamethylene amiloride, dimethyl amiloride, ethyl isopropyl amiloride and methyl isobutyl amiloride.

56. The preservation solution according to Claim 54, wherein said amiloride-containing compound is dimethyl amiloride.

57. The method according to Claim 54, wherein said cardioplegic solution comprises, per liter of solution, a balanced isotonic solution comprising sodium, potassium, calcium, and magnesium ions and bicarbonate in a physiologically acceptable amount, at least 0.5 μM of an amiloride-containing compound, and water.

58. The method according to Claim 54, wherein said perfusing is carried out at a temperature between from 15°C. to 37°C.

59. The method according to Claim 57, wherein said isotonic solution comprises, per liter of solution:

NaCl	85 mM	to	145 mM
KCl	3 mM	to	30 mM
$CaCl_2$	0.5 mM	to	2.5 mM

KH_2PO_4	0.7 mM	to	1.3 mM
$MgSO_4$	0.9 mM	to	4.8 mM
$NaHCO_3$	15 mM	to	35 mM
Glucose	1.0 mM	to	50 mM
EDTA	0.5 mM	to	1.5 mM
Distilled, deionized water		q.s.	

60. The method according to Claim 54, wherein said perfusing is carried out at a temperature between from 15°C. to 37°C.

61. A method of transplantation of a mammalian heart, said method comprising:

arresting the heart prior to removal from a donor with a solution comprising, in one liter of solution:

NaCl	85 mM	to	145 mM
KCl	3 mM	to	30 mM
$CaCl_2$	0.5 mM	to	2.5 mM
KH_2PO_4	0.7 mM	to	1.3 mM
$MgSO_4$	0.9 mM	to	4.8 mM
$NaHCO_3$	15 mM	to	35 mM
Glucose	1.0 mM	to	50 mM
EDTA	0.5 mM	to	1.5 mM
Distilled, deionized water		q.s.	

and at least 0.5 μM of an amiloride-containing compound;

removing said heart from said donor; and

perfusing said heart with a preservation solution while storing said organ at a temperature between 15°C. and 37°C.

62. The method according to Claim 61, wherein said preservation solution comprises, per liter of solution:

NaCl	85 mM to 145 mM
KCl	3 mM to 8 mM
$CaCl_2$	1.0 mM to 2.0 mM
KH_2PO_4	0.7 mM to 1.3 mM
$MgSO_4$	0.9 mM to 4.8 mM
$NaHCO_3$	15 mM to 35 mM
Glucose	1.0 mM to 50 mM
EDTA	0.5 mM to 1.5 mM
Apoferritin	1 μg/L to 100 μg/L
Catalase	1 μg/L to 10 μg/L
Caprylic acid	10 ηM to 100 ηM
DMTU	1 ηM to 10 ηM
DPPD	1 ηM to 10 ηM
DMA	1.0 μM to 5.0 μM
Distilled, deionized water	q.s.

63. The method according to Claim 61, wherein said perfusing is carried out at a temperature between from 15°C. to 37°C.

64. A method for making a carioplegic solution comprising, per liter of solution:

(a) formulating a balanced isotonic solution comprising sodium, potassium, calcium, magnesium and bicarbonate ions in a physiologically acceptable amount;

(b) admixing at least 0.5 μM of an amiloride-containing compound; and

(c) adding water.

65. A method for making a preservation solution for storage and reperfusion of organs intended for implantation comprising, per liter of solution:

(a) formulating a balanced isotonic solution comprising sodium, potassium, calcium, magnesium, bicarbonate ions in a physiologically acceptable amount;

(b) admixing from about 1.0 μM to about 5.0 μM of an amiloride-containing compound; and

(c) adding water.

INTERNATIONAL SEARCH REPORT

Inte...tional application No.
PCT/US95/16065

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A01N 1/02
US CL :435/1, 283

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/1, 283

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CHEM ABSTRACTS, BIOSIS, DERWENT, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	The Journal of Heart and Lung Transplantation, Volume 11, Number 4, Part 1, issued July/August 1992, Galinanes et al., "Long-Term Hypothermic Storage of the Mammalian Heart for Transplantation: A Comparison of Three Cardioplegic Solutions", pages 624-635, see page 626.	1-3, 5, 7, 9-11, 13, 15-20, 3-25, 27, 29-30, 32-34, 36, 37, 39-46, 48-52, 54-58, 60, 63-65

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 13 FEBRUARY 1996	Date of mailing of the international search report 04 MAR 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer RALPH GITOMER Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/16065

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Circulation Research, Volume 66, Number 4, issued April 1990, Dennis et al., "Effects of Proton Buffering and of Amiloride Derivatives on Reperfusion Arrhythmias in Isolated Rat Hearts", pages 1156-1159, see abstract.	1-3, 5, 7, 9-11, 13, 15-20, 23-25, 27, 29, 30, 32-34, 36, 37, 39-46, 48-52, 54-58, 60, 63-65
Y	Journal of Molecular and Cellular Cardiology, Volume 25, issued 1993, Moffat et al., "Protective Effects of the Potent Na/H Exchange Inhibitor Methylisobutyl Amiloride Against Post-Ischemic Contractile Dysfunction in Rat and Guinea Pig Hearts", pages 959-971, see the abstract.	1-3, 5, 7, 9-11, 13, 15-20, 23-25, 27, 29, 30, 32-34, 36, 37, 39-46, 48-52, 54-58, 60, 63-65
Y	The Journal of Pharmacology and Experimental Therapeutics, Volume 265, Number 3, issued 1993, Pierce et al., "Modulation of Cardiac Performance by Amiloride and Several Selected Derivatives of Amiloride", pages 1280-1291, see page 1286 column 2.	1-3, 5, 7, 9-11, 13, 15-20, 23-25, 27, 29, 30, 32-34, 36, 37, 39,-46, 48-52, 54-58, 60, 63-65
Y	CA, A, 2,089,336 (LIVESEY ET AL.) 13 August 1993, see pages 14, 37, 45, 56.	4, 6, 8, 12, 14, 21, 22, 26, 28, 31, 35, 38, 47, 53, 59, 61, 62